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(54) Title: CYTOLYTIC BRADYKININ ANTAGONISTS

(57) Abstract

The present invention provides bradykinin antagonists effective to inhibit cancer cell growth. Also provided are methods of inhibiting lung cancer cell growth by administering a therapeutically effective amount of a dimerized bradykinin antagonist.

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CYTOLYTIC BRADYKININ ANTAGONISTS

BACKGROUND OF THE INVENTION

Bradykinin (BK) is a potent inflammatory peptide whose generation in tissues and body fluids elicits many physiological responses including vasodilation, smooth muscle spasm, edema, as well as pain and hyperalgesia (Burch et al., "Molecular Biology and Pharmacology of Bradykinin Receptors", Landes Comp. (1993); Burch, edited: "Bradykinin Antagonists", Dekker (1991)). There is increasing evidence that BK and related kinins contribute to the inflammatory response in acute and chronic diseases including allergic reactions, arthritis, asthma, sepsis, viral rhinitis, and inflammatory bowel disease. Recently BK was implied to be involved as an autocrine in the pathogenesis of human lung cancer (Bunn et al., *Proc Natl. Acad. Sci. USA* 87:2162-2166 (1990); Bunn et al., *Cancer Research* 52:24-31 (1992)). BK has been shown to be the most potent peptide stimulant of intracellular Ca^{++} release in the highest fraction of human lung cancer cell lines (Bunn et al., *Cancer Research* 52:24-31 (1992)). The design and synthesis of specific, potent and stable bradykinin antagonists (BKA) has long been considered a desirable goal in medicinal chemistry. In the past few years, efforts have been directed towards the development of potent BK antagonists as a means for the chemoprevention and therapeutic treatment of human lung cancers.

Lung cancer is the second most common and the most lethal cancer in the United States. A large fraction of lung cancers (all small cell lung cancers (SCLC), some adenocarcinomas and a few squamous carcinomas) have a neuroendocrine phenotype (Becker et al., "The Endocrine Lung in Health & Disease", Saunders (1984)). These cancers and their premalignant precursors utilize a neuropeptide autocrine/paracrine growth factor pathway, i.e., they produce a variety of neuropeptides, express cell surface receptors for these peptides, and show autocrine stimulation by these peptides. Over the years, a number of specific and potent neuropeptide antagonists (including bradykinin, bombesin, cholecystokinin and many others) and anti-peptide antibodies were

developed and used in an attempt to inhibit the growth of the lung cancer cells which expressed receptors for these specific neuropeptides (Bunn et al., *Cancer Research* 54:3602-3610 (1994)). However, this approach failed to inhibit a majority of lung cancer cells because of the heterogeneity of neuropeptide receptor expression among the lung cancer cells.

It has been shown that broad spectrum substance P derivatives inhibited the growth of several lung cancer cell lines. However, very high concentrations (>40 μM) of these compounds were required, presumably because this interference occurs at the downstream level of the signal pathway (Bunn et al., *Cancer Research* 54:3602-3610 (1994)). It is thus desirable to provide neuropeptide antagonists with improved potency and specificity.

SUMMARY OF THE INVENTION

The present invention provides bradykinin (BK) antagonist dimers capable of inhibiting cancer cell growth. These dimers are generally described by the formula:

$$BKA_1\text{-}X\text{-}BKA_2, \quad (I)$$

wherein BKA_1 and BKA_2 are bradykinin antagonists and X is a linker group. BKA_2 is optionally absent (Formula II) thus providing an effective bradykinin antagonist comprising a BKA monomer and a linker.

Further provided, and also effective in inhibiting cancer cell growth, are compounds comprising a bradykinin antagonist and a neurokinin receptor antagonist according to the formula:

$$BKA\text{-}X\text{-}Y; \quad (III)$$

wherein BKA is a bradykinin antagonist peptide;

X is a linker; and

Y is neurokinin receptor antagonist.

In addition, the present invention provides dimerized neurokinin receptor antagonists:

$$Y_1\text{-}X\text{-}Y_2 \quad (IV)$$

wherein Y_1 and Y_2 are the same or different neurokinin receptor antagonists.

The invention further provides oligomers comprising 3 or more BKA's of the formula

$$(BKA)_n\text{-}X \quad (V)$$

where n is a whole number greater than 2.

Also provided by the invention are methods of inhibiting lung cancer cell growth by administering to a subject afflicted with lung cancer, a therapeutically effective amount of one or more of the compounds according to Formulas I, II, III, IV or V.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the effect of compounds B196, B197 and B198 on SCLC growth.

Figure 2 shows the effect of compounds B199, B200 and B201on SCLC growth.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that certain dimerized bradykinin antagonist peptides are highly effective in inhibiting the growth of cancer cells, particularly lung cancer cells, i.e., small cell lung sarcinoma. These dimers comprise antagonists which are analogs of the bradykinin peptide (Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9). A majority of antagonists known in the art represent modifications whereby DPhe has been substituted with LPro in position 7 of the BK sequence and are selective against the B2 class of BK receptor, which is expressed in most of the human lung cancer cell lines. Many of these analogs, with or without pseudo-peptide bond modifications, were found to be specific and stable but only exhibited moderate potency in calcium flux assays. It has been found by the present inventors that a series of BKA dimers based on the classical B2 receptor antagonists with DPhe substitution (Cheronis et al., *J. Med. Chem.* 35:1563-1572 (1992)) have improved potency and stability.

A third generation of BK antagonists (Burch et al., "Molecular Biology and Pharmacology of Bradykinin Receptors", Landes Comp. (1993)) containing modified amino acids (Tic, Oic, Igl, Nig) in positions 5, 7, and 8 of BK's primary sequence were synthesized and found to be several orders of magnitude

more potent B2 receptor antagonists than the classical DPhe7 substituted analogues in various assays *in vitro*. Unfortunately, even at very high concentrations all of these BK antagonists failed to show any growth inhibitory effects in the human lung cancer cell lines, presumably due to the receptor heterogeneity.

A further class of BK antagonist dimers were synthesized by cross linking the third generation BK antagonists. This modification not only increased the potency and stability of these B2 receptor antagonists, but several of this new class of antagonists were able to inhibit the growth of human lung cancer cells completely even at concentrations of 10 μM or less. Furthermore, preliminary studies have shown that lung cancer cell lines are more sensitive to the cytotoxic effects of these new antagonists than those of normal epithelial and fibroblast cell lines. It has also been found that these new antagonists induce apoptosis in the treated lung cancer cells.

The dimers may be represented by the formula

$$BKA_1\text{-}X\text{-}BKA_2, \quad \text{(I)}$$

wherein BKA_1 and BKA_2 are bradykinin antagonists and X is a linker. Preferably, BKA_1 and BKA_2 are independently selected from the following:

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (SEQ ID NO:1);
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
ε-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Gun-Gly-ε-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:2);
Dhq-DArg-Arg-Pro-Hyp-Gly-ϵ-Lys-Ser-DCpg-CPg-Arg;
Dhq-ϵ-Lys-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg-Arg;
DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg;
DArg-Cys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;
DArg-Lys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;
DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;
Gun- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;
Gun- DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;
Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;
Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
Lys- Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic; and
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic.

The linker X may be any linking group which does not interfere with the inhibitory activity of the monomer-linker or dimerized product using ester, imido-ester, and thio-ester based linking agents, for example. X may be an N-terminal acylating or cross-linking group including a bissuccinimidoalkane such as bissuccinimidohexane; bissuccinimidoalkene; bissuccinimidoamine; bis(imidyl)alkenyl or -alkyl such as suberimidyl; aminocaproic acid-succinyl; dicarboxylic acid derivatives such as succinyl and suberyl; epsilon-succinimido-N-caproyl; and methoxy-suberimido-based linker. The alkane groups may be substituted with, for example, carbonyl and/or amino groups. Polyoxyethylene linkers may also be used.

With regard to linker length, there appears to be a correlation between linker length and cytotoxicity, i.e., the longer the linker, the higher the potency of the compound. Therefore, the linker may comprise alkyl chains 6 carbons in length or greater. Alkyl chains of 8 carbons or more are preferred, with those of 12 to 18 carbons being most preferred. Chain lengths of greater than 18 carbons may also be used. Examples of such preferred linker groups include

bissuccinimidohexane, bissuccinimidooctane, bissuccinimidononane and bissuccinimidodecane.

The monomers may be linked at any position of the BKA. For example, linking may be achieved via the N-terminus, either through the terminal arginine or through an added lysine residue. Alternatively, serine, if present, may be substituted with cysteine or lysine for internal linkage via the S or N of the side chain, respectively. It is preferred that there be at least one basic charge at the amino end of the dimerized or monomer-linker compounds. For example, the charge may be on the amino group of an N-terminal lysine residue or on the imide group of the linker.

In a particular embodiment, the bradykinin antagonist dimer is selected from:

B2120 Suc-(Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)$_2$

B2124 Suc-(Eac-Eac-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)$_2$

B9830 Suim-(DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg)$_2$

B9832 Sub-(DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg)$_2$

B9836 BSH-(S-Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)$_2$

CP-0127 DArg-Arg-Pro-Hyp-Gly-Phe-Cys-DPhe-Leu-Arg-COOH
|
BSH
|
DArg-Arg-Pro-Hyp-Gly-Phe-Cys-DPhe-Leu-Arg-COOH

B168 Eac-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic-Arg.TFA
|
Suc-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic-Arg.TFA

B196 DArg-Arg-Pro-Hyp-Gly-Igl-Cys-DIgl-Oic-Arg.TFA

|

DArg-Arg-Pro-Hyp-Gly-Igl-Cys-DIgl-Oic-Arg.TFA;

B197 Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA

|

BSH

|

Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA;

B198 Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA

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SUB

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Gun-Gly-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-desArg-COOH;

B199 Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA

|

SUB

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Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA

B201 DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA

|

SUIM

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DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA

B204 DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.TFA

|

SUIM

|

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.TFA

B2122 Suc-(Eac-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)$_2$;

Dhq-DArg-Arg-Pro-Hyp-Gly-Lys-Ser-DCpg-CPg-Arg

|

SUC

|

Dhq-DArg-Arg-Pro-Hyp-Gly-Lys-Ser-DCpg-CPg-Arg; and

B9878 (HPLC3) Suim-(DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg)$_2$,

where

BMH = Bismaleimidohexane

EAC-SUC = Aminocaproic acid-succinyl

SUB = Suberyl

SUIM = Suberimidyl

MOSI = Methoxy-suberimido

BSH = Bissuccinimidohexane

ESC = Epsilon-Succinimido-N-Caproyl

TFA = Trifluoroacetic acid

SUC = Succinyl.

In a more preferred embodiment, BKA$_1$ and BKA$_2$ are selected from

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg, and

Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.

Preferably, BKA$_1$-X-BKA$_2$ is

Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg

|

SUB

|

Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg; or

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg

|

SUIM

|

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.

The present invention also provides compounds of the formula

BKA-X, (II)

where BKA and X are as previously described.

Preferred compounds according to Formula II include

MOSI-Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

MOSI-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg; and

MOSI-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.

Further provided are compounds of the formula

BKA-X-Y, (III)

wherein BKA is a bradykinin antagonist peptide;

X is a linker; and

Y is a neurokinin receptor antagonist.

Any neurokinin receptor antagonist known in the art may be used in these heterodimeric embodiments. Such antagonists are described, for example, in Langdon et al., *Cancer Research* 52: 4554-4557 (1992); Orosz et al., *Int. J. Cancer* 60:82-87 (1995); and Woll et al., *Cancer Research* 50:3968-3973 (1990). Some of these monomeric antagonists have demonstrated inhibitory activity against small cell lung cancer.

By way of example, Y may be selected from

Asp-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$;

Cys-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$;

DArg-DArg-Lys-Pro-Lys-Asn-DPhe-Phe-DTrp-Leu- (Nle);

p-HOPA-DTrp-Phe-DTrp-Leu-NH_2;

p-HOPA-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DMePhe-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DMePhe-DTrp-Tyr-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DTyr(Et)-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DPhe-DTrp-Phe-DTrp-Leu-OH;

DMePhe-DTrp-Phe-DTrp-Leu-OH;

DPhe-DTrp-Phe-DTrp-Leu-MPA;

DMePhe-DTrp-Phe-DTrp-Leu-MPA;

DTyr-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2; and

DMePhe-DTrp-Phe-DTrp-Leu-Leu-NH_2.

where

HOPA = para-hydroxy-phenyl-acetic group

DMePhe = D-N-methyl-phenylalanine

MPA = 2-amino-methylpentane.

In a preferred embodiment of Formula III, BKA is selected from

Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg; or

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg; and

Y is DMePhe-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2.

Compounds of Formula III may be linked via any known method. For example, where both the BKA and the Y component are peptides, they may be linked via their N-terminals. Using the bis(imidoester) crosslinking agent dimethyl suberimidate, the following heterodimer may be synthesized:

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg

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SUIM

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DPhe-DTrp-Phe-DTrp-Leu-OH.

Other examples of bis(imidoester) linking agents which may be used include dimethyl butyl-, -octyl-, -decyl-, -dodecyl-, and -tetradecylimidate, although any of the above described linker groups X may be used.

Further provided are dimerized neurokinin receptor antagonists of the formula

$$Y_1\text{-}X\text{-}Y_2 \quad \text{(IV)}$$

where Y_1 and Y_2 are the same or different neurokinin receptor antagonists, as defined herein for Y. Several methods of linking these Y components are apparent from the disclosure and other methods will be known to those of skill in the art.

In addition, compounds of the formula

$$(BKA)_n\text{-}X \quad \text{(V)}$$

where n is a whole number greater than 2, are also effective inhibitors of cancer cell growth. In a preferred embodiment, n is 3. Any of the X groups described herein may be modified for linking the trimers. Examples of linkages which may be used where n is 3, i.e., a trimer, include trissuccinimidoalkane and trissuccinimidoamide. Procedures for their preparation are described in Cheronis et al., *J. Med. Chem.* 35:1563-1572 (1992)).

Also described are methods of inhibiting lung cancer cell growth through administration of one or more of the compounds according to Formulas (I through V).

In a preferred method of inhibition, BKA_1, BKA_2 and BKA are peptides, preferably, selected from

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (SEQ ID NO:1);
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
ε-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Gun-Gly-ε-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:2);

Dhq-DArg-Arg-Pro-Hyp-Gly-ϵ-Lys-Ser-DCpg-CPg-Arg;

Dhq-ϵ-Lys-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg-Arg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg;

DArg-Cys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

DArg-Lys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;

Gun- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;

Gun- DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;

Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;

Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

Lys- Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Phe-Cys-DPhe-Leu-Arg; and

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.

In a particularly preferred embodiment, BKA is

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

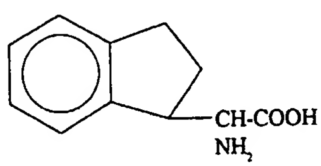
ε-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic; or

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.

As described above, it is preferred that the dimers be comprised of modified bradykinin antagonist peptides that contain amino acids substituted on the α-carbon or on the α-nitrogen by 1-indanyl or 2-indanyl groups. These monomers are described in U.S. Patent Application Serial No. 08/344,636. In a preferred embodiment, the bradykinin

antagonist peptide monomers contain indane-substituted amino acid residues at positions five, seven and eight of the bradykinin native sequence. According to this invention, the indane substituent can be on either the α-carbon (residues abbreviated Igl) or the nitrogen (residues abbreviated Nig) of the glycine residue, and the indane residue can be attached to the glycine moiety at either position 1 (Igla or Niga) or position 2 (Iglb or Nigb) of the indane group.



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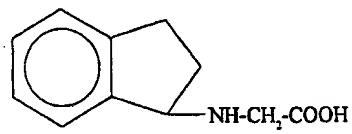
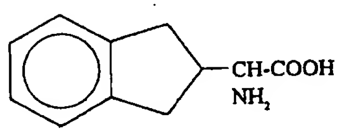
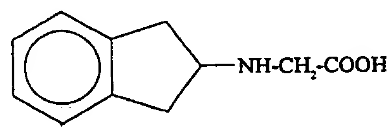


Niga



Iglb



Nigb

As used herein, abbreviations of the natural amino acids are those accepted in the art (*Biochem. J.* 126: 773 (1972)), and unless prefixed with D are all of the L-configuration (except glycine and MPIV, which are not optically active).

Abbreviations used for unnatural amino acids in Bradykinin analogs are indicated below:

AC6	1-Aminocyclohexane-1-carboxylic acid
Alg	Allylglycine
Azt	Azetine-2-carboxylic acid (norproline)
CDF	*p*-Chloro-D-Phe
Chg	CyclohexylGly (α-Aminocyclohexaneacetic acid)
cLeu	1-Aminocyclopentane-1-carboxylic acid (cycloleucine)
Cpg	CyclopentylGly (α-Aminocyclopentaneacetic acid)

Dhp	3,4-Dehydro-Pro
DMF	2,4-Dimethylphenylalanine
Eac	6-Aminohexanoic acid (ε-aminocaproic acid)
FDF	*p*-Fluoro-DPhe
Gun	Guanidyl
HBQ	N5-(4-hydroxybutyl)-glutamine
Hig	Hexahydroindanylglycine
Hyp	*trans*-4-Hydroxy-Pro
Igla	α-(1-indanyl)glycine
Iglb	α-(2-indanyl)glycine
Inip	Isonipecotic acid (piperidine-4-carboxylic acid)
MDY	O-Methyl-DTyr
MPIV	2,4-Methanoproline (2-Azabicyclo-(2,1,1)-hexane-1-carboxylic acid)
Nal	b-2-Napthyl-Ala
NChg	N-substituted cyclohexylglycine
Niga	N-(1-indanyl)glycine
Nigb	N-(2-indanyl)glycine
Nle	Norleucine
NMF	N-Methylphenylalanine
Oic	Octahydroindole-2-carboxylic acid
OMT	O-Methyl-Tyr
Pal	b-3-Pyridyl-Ala
PCF	*p*-Chloro-Phe
Pip	Pipecolic acid ("homo-Pro")
Pop	*trans*-4-PropoxyPro
Ser(SO_4)	Serine-O-sulfate
Suc	Succinyl
Thi	β-2-Thienyl-Ala
Thz	Thiazolidine-4-carboxylic acid
Tic	1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid

Abbreviations used for acylating groups, in addition to those described above, are as follows:

Aaa-	1-Adamantaneacetyl-
Ac-	Acetyl-
Aca-	1-Adamantanecarbonyl-
Bz-	Benzoyl-
Cha-	Cyclohexaneacetyl-
Cpa-	Cyclopentaneacetyl-
Dca-	2,2-Dicyclohexylacetyl-
Dhq-	2,3-Dehydroquinuclidine-3-carbonyl-
Dpa-	2,2-Diphenylacetyl-
Dpp-	3,3-Diphenylpropionyl-
Nba-	Norbornane-2-acetyl-
Nbc-	2-(*cis*-5-norbornene-*endo*-3-carbonyl)-
Nbi-	*cis*-5-norbornene-*endo*-2,3-dicarboximidyl-
Paa-	Phenylacetyl-
Pba-	4-Phenylbutyryl-
Ppa-	3-Phenylpropionyl-
Sin-	Sinapinyl- (3,5-dimethoxy-4-hydroxycinnamyl-)

The description of peptide synthesis methods uses several abbreviations for standard solvents, reagents and procedures, defined as follows:

BOP	Benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluorophosphate
BuOH	n-Butanol
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIC	Diisopropylcarbodiimide
DIEA	Diisopropylethyl amine
DMF	Dimethylformamide
HATU	O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HOAc	Acetic acid
MeOH	Methanol
OHMR	Hydroxymethylpolystyrene resin for peptide synthesis, 1% crosslinked.
TBTU	O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TEA	Triethyl amine
TFA	Trifluoroacetic acid

The following abbreviations for blocking groups used in synthesis are:

Boc	*t*-Butyloxycarbonyl
Tos	*p*-Toluenesulfonyl
Bzl	Benzyl ether

The following abbreviations for standard techniques used are:

AAA	Amino acid analysis (Stewart & Young p. 108)
CCD	Countercurrent distribution (Stewart & Young p. 96)
ELEC	Paper electrophoresis (Stewart & Young p. 117)
HPLC	High performance liquid chromatography (Stewart & Young, p. 100)
Kaiser test	Ninhydrin test for completeness of coupling reactions (Stewart & Young, p. 105)
SPPS	Solid phase peptide synthesis
TLC	Thin-layer chromatography (Stewart & Young, p. 103)

The synthesis of peptides described herein, including preparation of appropriate amino acid derivatives, their activation and coupling to form peptides and methods for purification of peptides and determination of their purity are included in the general body of knowledge of peptide chemistry, as generally described in Houben-Weyl "Methoden der Organischen Chemie". Vol. 16, parts I & II, (1974) for solution-phase synthesis, and in "Solid Phase Peptide Synthesis" by Stewart and Young (1984) for synthesis by the solid phase method. A chemist skilled in the art of peptide synthesis would be able to

synthesize the described peptides by standard solution methods or by manual or automatic solid phase methods.

In a majority of cases there appears to be a correlation between BK antagonist potency and the ability to inhibit cell growth. Therefore, it may be desirable to screen monomeric components for BK antagonism prior to dimerization as an indication of potential inhibitory action.

To determine bradykinin antagonist activity, the bradykinin antagonists may be assayed on isolated rat uterus in natural or induced estrus and on guinea pig ileum, according to the commonly accepted assay methods for bradykinin and related kinins as described by Trautschold (Handbook of Experimental Pharmacology, Vol. 25, Springer-Verlag, pp 53-55, (1969)) for inhibition of the myotropic activity of bradykinin. The inhibition potencies may be determined according to the commonly accepted manner, as described by Schild for antagonists of biologically active compounds (*Brit. J. Pharmacol.* 2: 189 (1947)) and expressed as pA_2 values. In the assays, a dose-response curve is determined for the reference substance bradykinin. The dose of bradykinin which produces a half-maximal contraction of the tissue is the ED_{50} dose. An amount of bradykinin equivalent to twice the ED_{50} dose is administered to the tissue 30 seconds after the start of incubation of the tissue with a dose of antagonist. Doses of antagonist are increased in this protocol until the dose of antagonist is found which causes the tissue response to a double ED_{50} dose of bradykinin in the presence of antagonist to equal the response of an ED_{50} dose of bradykinin without antagonist. The pA_2 value represents the negative logarithm of the molar concentration of antagonist necessary to reduce the response to a double ED_{50} dose of bradykinin to that of an ED_{50} dose without antagonist. A change of one unit of pA_2 value represents an order of magnitude change in potency. For comparison, the negative logarithm of the dose of bradykinin that causes half-maximal contraction of the tissues, commonly known as the pD_2 value, is 7.9 on the rat uterus and 7.4 on the guinea pig ileum.

Binding assays using BK1, BK2 human receptor clones or BK2 guinea pig ileum smooth muscle membrane receptor preparations may also be used to screen potential components.

With regard to the heterodimeric compounds, i.e., those of the formula BKA-X-Y, and the dimeric NK1 and NK2 antagonists (Y_1-X-Y_2), the antagonistic properties of the neurokinin antagonist can be determined through assay methods well known in the art.

The effectiveness of the antagonist compounds in inhibiting cancer cell growth *in vitro* was determined using a panel of human lung cancer cell lines: SCLC--SHP77, H345 and NSCLC--A549, H450; breast carcinoma McF7, normal human epithelial BEAS and normal human skin fibroblast FS15 were used to study the specificity and cytotoxicity of the BK antagonist dimers. Cell lines were treated with various concentrations of dimers for 5 to 7 days and cell viability were measured with routine MTT assay. Cells treated with BK dimers were also be evaluated for apoptosis using our newly developed cytometric technique.

The *in vivo* inhibitory effects of antagonists may be studied using tumor-bearing nude mice. A tumor model employing nude mice orthotopically implanted with human lung cancer cells wherein the antagonists are delivered by intratracheal instillation and aerosol inhalation may be used to evaluate the efficacy and feasibility of these antagonists as a means of treating human lung cancers. Control animals without tumor implantation may also be used to study the general side effects or cytotoxicity of the compounds. It is believed that aerosolized delivery or intratracheal instillation of the agents can produce effective dose accumulation in the area of lesion and reduce the overall systemic toxicity of the compounds in the animals than when the compound is delivered by intravenous injection.

Aerosolized BK antagonists have been used to treat animals with airway hyperreactivity. Thus, the study may allow evaluation of the possibility of using this kind of BK antagonist dimers for treating human pulmonary diseases such as asthma and other inflammatory diseases.

In addition to the above described embodiments, the following compounds are also disclosed herein (the monomer-linkers, dimers and trimers are linked via the available cysteine residue, unless otherwise indicated):

CP-0088 DArg-Arg-Pro-Hyp-Gly-Phe-Ser-DPhe-Leu-Arg

CP126 [Cys^6]-CP-0088 or also DArg-Arg-Pro-Hyp-Gly-Phe-Cys-DPhe-Leu-Arg

CP127 N-hexyl succinimido - $(CP126)_2$

CP162 Bissuccinimidoethane - $(CP126)_2$

CP166 Bissuccinimidododecane - $(CP126)_2$

CP172 Bissuccinimidopropane - $(CP126)_2$

CP174 BSH - CP126

CP211 Bissuccinimidooctane - $(CP126)_2$

CP229 Bissuccinimidononane - $(CP126)_2$

CP230 Bissuccinimidodecane - $(CP126)_2$

CP360 BSH - $(DArg\text{-}Arg\text{-}Pro\text{-}Hyp\text{-}Gly\text{-}Ala\text{-}Cys\text{-}DAla\text{-}Ala\text{-}Arg)_2$

CP394 CP126 - (ESC) - Lys^5 (CT008)

CP397 CP126 - (ESC) - Lys^8 (CT008)

CP411 CP126 - (BSH) - Cys^1 (CT0022)

CP597 DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-NChg-Arg

CT008 DArg-DPro-Lys-Pro-Gln-Asn-DPhe-Phe-DTrp-Leu-Nle-$CONH_2$

CT0022 Asp-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$

B9810 Gun-Gly-ε-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:2)

B9878 MOSI-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg

CP352 DArg-Arg-Pro-Hyp-Gly-Thi-Cys-DTic-Oic-Arg
|
BSH
|
DArg-Arg-Pro-Hyp-Gly-Thi-Cys-DTic-Oic

CP164 Tris[2-γ-succinimidobutyramido)ethyl]amine - $(CP126)_3$

CP171 $HO\text{-}CH_2\text{-}C\text{-}[(CH_2OC(O)CH_2CH_2\text{-}(CP126)]_3$

The compounds may be administered topically, or by injection or infusion or as an oral suspension in an appropriate vehicle or as tablets, pills, capsules, caplets or the like, or preferably via intratracheal instillation or aerosol inhalation. The dosage and manner of administration will be defined by the application of the bradykinin antagonist and can be determined by routine methods of clinical testing to find the optimum dose. These doses are expected to be in the range of 0.001 mg/Kg to 100 mg/Kg of active compound.

The compounds are composed of amino acids which may form salts due to their acidic or basic nature, and any pharmacologically acceptable salt derived from the compounds described in this invention such as hydrochlorides, acetates, phosphates, maleates, citrates, benzoates, salicylates, succinates, ascorbates and the like, including HCl, trifluoroacetic acid (TFA), and HOAc, are considered an extension of this invention. A common tactic in medicinal chemistry is to modify known drug substances which are peptide based to form esters or amides which exhibit greater bioavailability. Prodrugs derived from the compounds disclosed here are therefore considered an obvious extension of this invention. Methods for designing and preparing prodrugs are described in detail in the medicinal chemical literature.

EXAMPLES

EXAMPLE I - Synthesis of dimers of Bradykinin Antagonists

Dimers were synthesized on either solid phase resin support or in solution.

Succinyl-bis-peptide dimers on resin (B9132 and B9572 (B168)

A ten-fold excess of succinic anhydride with a ten-fold excess of triethylamine was allowed to react with the peptide-resin to give the succinyl peptide (mono-adduct) on the peptide-resin. Then the pure, neutralized peptide monomer was allowed to react in DMF with the BOP-, TBTU-, or HATU-activated succinyl-peptide on the resin to give the succinyl-bis peptide dimer still attached to the resin. The finished peptide-dimers were cleaved form the resin using standard HF procedures (Stewart et al., "Laboratory Techniques in Solid-Phase Peptide Synthesis" in Solid-Phase Peptide Synthesis, 2nd ed., Pierce

Chemical Co., Rockford, IL, pp. 71-72 (1984)). The free peptides were extracted with acetic acid, lyophilized and purified with reverse-phase HPLC.

Suberyl-bis-peptide dimers on resin (B9860 HPLC#3 and HPLC#4)

A five-fold excess of dissuccinimidyl suberate (DDS) with a 1-2 fold excess on N,N-diisopropyl-ethylamine (DIEA) in DMF was allowed to react with the peptide resin having a free ϵ -Lys group (B9810) was allowed to react with the suberyl-peptide on the resin to give the Sub-bis-peptide dimer still attached to the resin. The finished peptides were cleaved from the resin using standard HF procedures (Stewart). Free peptides were extracted with acetic acid, lyophilized and purified by preparative reversed-phase HPLC. The HPLC separation gave hetero- (B9860HPLC#3) and homo-dimer (B9860 HPLC#4).

Suberyl-bis-peptide dimers in solution (B9832)

One equivalent of neutralized peptide with 10 equivalents of DIEA and 0.75-1 equivalents of disuccinimidyl suberate (DSS) were allowed to react overnight in DMF at room temperature, and the resulting dimer was purified by preparative reversed-phase HPLC.

Bis-succinimidohexane Peptide Dimers in Solution (B9834 and B9836)

One equivalent of Cys-containing peptide monomer salt, ten equivalents of DIEA and 0.75 equivalents of bismaleimidohexane (BMH) linker were allowed to react overnight in DMF. The resulting vissuccinimidoalkane peptide dimers were purified by preparative reversed-phase HPLC.

Using the same chemistry, dimers were synthesized with longer linkers:

BSO = bissuccinimidooctane

BSN = bissuccinimidononane

BSD = bissuccinimidodecane.

Suberimidyl-bis-peptide dimers in solution (B9830, B9870, B9872, B9878)

One equivalent of peptide monomer salt, 15 equivalents of DIEA and 1 equivalent of dimethyl suberimidate.2HCl (DMS) were stirred overnight in DMF at room temperature. The DMF was removed in vacuo and the residue was purified by reversed-phase HPLC. The HPLC separation gave the MOSI-monomers and the SUIM-bis-dimers generally in a 1:2 ratio.

Using the same chemistry, dimers were synthesized with longer linkers using the following linking agents:

Dimethyl sebacimidate (10-carbon linker)

Dimethyl decanedicarboximidate (12-carbon linker)

Dimethyl dodecanedicarboximidate (14-carbon linker).

EXAMPLE II - Cys-(Succinimido-*N*-Caproyl)-Lys-BK2/NK1 Antagonist Heterodimers

To a preparation of L-Lys(Fmoc)-containing NK_1 peptide antagonist-resin (MBHA, 0.5 mmole peptide) was added 50% piperidine in DMF (ca. 20 mL). The resulting mixture was bubbled gently with N_2 (g) for 20 minutes to afford complete removal of the Lys(Fmoc) protecting group and then the peptide-resin was washed well with DMF. The peptide-resin was resuspended in DMF and 1.5 equivalents of EMCS (epsilon-maleimido-*n*-caproic acid *N*-hydroxysuccinimide ester) were added. The acylation reaction was allowed to proceed at room temperature for 2-3 hours (verification of complete acylation accomplished with the Kaiser test) after which time the peptide-resin was washed well with DMF, then with 10% (v/v) NH_4HCO_3/DMF (NH_4HCO_3 stock concentration: 0.1 M, pH 8). The BK_2 antagonist CP126, 3 equivalents in 10% (v/v) NH_4HCO_3/DMF was added to the maleimido-containing peptide-resin and the subsequent conjugate addition (1,4-addition or Michael reaction) allowed to proceed at room temperature for several hours. The peptide-resin was then washed well (successively) with 10% (v/v) NH_4HCO_3/DMF, DMF and dichloromethane. Following extensive drying *in vacuo*, the Cys-(succinimido-*n*-caproyl)-Lys BK_2/NK_1 antagonist heterodimer was deprotected/cleaved from the peptide-resin with anhydrous HF at 0°C and then purified by preparative reversed -phase HPLC. Lyophilization afforded the pure peptide in 50-60% yield as a fluffy, white powder.

EXAMPLE III - Cys-[Bis(Succinimido)Hexane])-Cys-BK2/NK2 Antagonist Heterodimers

To a mixture of Cys-containing NK_2 peptide antagonist (1 equivalent) and *bis*(malameimido)hexane (BMH, 2 equivalents) in DMF (ca. 21 mL per

mmole of peptide) was added 10 volumes of NH_4HCO_3 (pH 8). The reaction mixture was stirred at room temperature for several hours (monitored periodically by analytical reversed-phase HPLC) and the resulting *S*-[(*N*-hexyllmaleimido)succinimido] derivative of the NK_2 antagonist purified by preparative reversed-phase HPLC. The resulting peptide, isolated in approximately 70% yield after lyophilization, was then combined with the BK_2 antagonist CP126 (1.5 equivalents) in DMF (same mL/mmole as specified above). Ten volumes of NH_4HCO_3 (pH 8) were added and the dimerization allowed to proceed for several hours at room temperature. The resulting Cys-[*bis*(Succinimido)Hexane])-Cys BK2/NK2 antagonist heterodimer was purified by preparative reversed-phase HPLC and lyophilized to yield a white, fluffy powder. OVerall yields ranged from 40-50%.

EXAMPLE IV - BK1/BK2 Binding Assays

Human lung fibroblasts IMR-90 cells were obtained from ATCC and propagated in DMEM media in 850 mm roller bottles until confluent. Three hours prior to harvesting, the cells were treated with Interleukin lb (200 pg/ml). Human BK2 clones were propagated in F12 media until confluent. Preparation of membranes for binding assays was carried out by scraping cells from roller bottles in ice cold PBS and centrifuging at 1000 xg, at 4°C for 15 minutes. The supernatant was discarded and pellet resuspended in Buffer A consisting of 25 mM TES(pH 6.8) with 2 uM 1,10-Phenanthroline, and centrifuged at 27,000 xg for 15 min. this was then repeated. The final pellet was resuspended in Buffer B (Buffer A with 2 uM Captopril, 140 ug/Ml Bacitracin, 0.1%BSA), and stored in 1 ml aliquots, frozen at -20°C until needed.

Binding assays were performed by incubating human clone membranes with 0.3 nM ^{3}H-Bradykinin or IMR-90 membranes with 0.5 nM ^{3}H-des-Arg^9-Kallidin in the presence of the peptides in assay buffer (Buffer B with 1 mM Dithiotreitol), at room temperature, for 45 minutes. All test compound dilutions were in triplicate. Assays were harvested by quick filtration in a Tomtec Harvester 96, with ice cold wash buffer consisting of 10 mM Tris/HCl, pH 7.5, 100 mM NaCl, .02%BSA, onto Wallec printed glassfiber Filtermat "B", which

had been pre-soaked with 0.1% PEI and previously air-dried. Filtermats were counted in 9.5 mls Wallec Beta-Plate Scint, in Wallec 1450 MicroBeta Counter.

Results are shown in Table I

TABLE I

Receptor binding data - Human B1/B2; GPI B2

Compound	Binding: Human B1 Receptor - pIC_{50}	Binding: Human B2 Receptor - pIC_{50}	Binding: GPI (B2) pIC_{50}
B9572		8.7	8.5
B9830	6	8.3	8.6
B9832	6	8.4	8.9
B9834	7.2	8.4	7.9
B9836	7.9	9.1	8.8
B9860 (HPLC3)	8.5	9.3	9
B9860 (HPLC4)	8.0	8.8	8.5
B9870 (HPLC1)	7.8	9.1	9.1
B9870 (HPLC2)	7.9	8.4	8.3
B9872 (HPLC2)	NT	9.5	9.7
B9872 (HPLC3)	NT	8.8	9.0
B9878 (HPLC2)	8.5	6.4	6.1
B9878 (HPLC3)	8.9	6.5	6.5

pIC_{50}= -log of the IC_{50} (concentration in molar which inhibits tritiated ligand binding by 50%)

EXAMPLE V - Rat Uterus Assay for B2 Receptor for B2/NK1 and B2/NK2 Comp unds

Female rats were pretreated with stilboestrol, 100 ug/kg s.c. Eighteen hours later the animals were sacrificed by CO_2 asphyxiation. The uterine horns

were removed and attached to tissue holders which were placed in 5 ml tissue baths containing DeJalon's solution at 31°C and bubbled with air. Tissues were placed under 1 m isometric resting tension. Cumulative concentration effect curves were constructed to bradykinin in the absence and presence of increasing concentrations of the antagonist at 1 h intervals. pA_2 values were calculated according to the method of Schild .

EXAMPLE VI - NK1 Assay for BK/NK1 Compounds - Guinea Pig Ileum

Male guinea-pigs were sacrificed by CO_2 asphyxiation. The ileum was removed and cleaned of fat and mesenteric tissue. Longitudinal sections, 25 mm in length were attached to tissue holders and placed in 5 ml tissue baths containing Kreb's solution at 37°C and bubbled with $95\%O_2/5\%CO_2$. Tissues were placed under 2 gm isometric resting tension. Following a 1 h incubation period, cumulative concentration effect curves were constructed to substance P in the absence and presence of increasing concentrations of the antagonist at 1 h intervals. Compound CP394 yielded a pA_2 value of 5.8.

EXAMPLE VII - NK2 Assay for BK/NK2 Compounds - Rabbit Pulmonary Artery

Female New Zealand White Rabbits were sacrificed by CO_2 asphyxiation. The pulmonary artery was removed and prepared as vascular rings. The preparations were secured on tissue holders and placed under 1 gm isometric resting tension in 5 ml tissue bathes containing Krebs solution at 37°C and bubbled with $95\%O_2/5\%CO_2$. Cumulative concentration effect curves were constructed to neurokinin A in the absence and presence of increasing concentrations of the antagonists. Compound CP411 yielded a pA_2 value of 5.5.

EXAMPLE VIII - Calcium Flux Assays

Cells were loaded with the calcium marker Indo-1 AM (Molecular Probes, Eugene, OR) according to the methods described previously (Bunn et al.: Proc. Natl. Acad. Sci. USA. Vol, 87, 2162-2166, 1990). Briefly, mechanically dispersed single cell suspensions of plateau phase cells (7 days after splitting) were suspended in 20 mM HEPES/BSS buffer (20 mM Hepes/140 mM NaCl/5 mM KCl/1 mM $MgCl_2$/5 mM Glucose) adjusted to pH 6.8 at 37°C. The

acetoxymethyl ester form of Indo-1 (Indo-AM) at 5 uM was incubated with the cells for 30 min at 37° C and then diluted with 1:1 with 20 mM HEPES/BSS buffer, pH 7.4 to raise the final pH to 7.1 followed by a second 30-min incubation at 37°C. The cells were then washed twice in HEPES/BSS buffer, pH 7.4 (37°C) containing dextrose and were resuspended to 1 x 10^6 cells per ml prior to flow cytometric analysis, which was performed with an EPICS 752 cell sorter (Coulter).

UV excitation (80 mW at 360 nm) was delivered by a model INNOVA 90/5 argon ion laser (Coherent, Palo Alto, CA). Violet emission fluorescence intensity (397-417 nm), which is emitted from calcium-bound indo-1, was obtained with a 410 band pass filter (Oriel, Stratford, CT) and blue emission fluorescence (480-500 nm), which is emitted from free indo-1, was obtained with a 490 band pass filter (Oriel). Thus, the ratio of the 410 nm fluorescence/490 nm fluorescence constitutes a measure of the changes in calcium ions liberated form intracellular stores (Endoplasmic reticulum) in response to various stimuli. Viable, loaded cells were distinguished by the machine form debris, dead cells, and unloaded cells by forward angle and 90° light scatter and 490 nm fluorescence. The ratio of 410 nm fluorescence /490 nm fluorescence emission was calculated digitally for each cell by the MDADS hardware and displayed on a linear scale. Data were collected by the MDADS computer including 410 nm and 490 nm fluorescence intensities and the ratio of 410/490 fluorescence intensity was secured as a function of time. For each experiment, measurements were first performed on unstimulated cells to establish the base line followed by the addition of each specific bradykinin antagonist first and then 3-5 min later followed by the bradykinin agonist. Cell flow was halted briefly (approximately 10 sec) for the administration of each peptide. The data were analyzed by a program developed by Philip Jewett.

EXAMPLE IX - Colorimetric MTT (tetrazolium) Assay

Cellular growth and survival was measured by a rapid colorimetric assay, based on the tetrazolium salt MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), developed by Mosmann (J. of Immun. Methods, 65, 55-

63, 1983) with minor modifications. Briefly, 1,000 normal lung fibroblasts or normal epithelial BEAS-2B cells, 1,000 or 5,000 viable NSCLC cell and 10,000 viable SCLC cell were plated in a 100 ul volume of growth medium in 96 well flat-bottomed microtiter plates. The cells were allowed to recover overnight. Bradykinin antagonists at various concentrations were added to the cells in triplicates and the plates were incubated at 37°C , 5% CO_2 with 100 % humidity. Control cells were treated in a same way without the antagonists. All wells had a final volume of 200 ul and the plates were incubated for 3-4 days allowing sufficient time for the cell replication and antagonist induced cell death to occur. On the 5th day, 25 ul of a 2 mg/ml solution of the tetrazolium salt MTT (Sigma Chemical CO, St. Louis, MO) dissolved in RPMI1640 was added to each well. The microtiter plate was incubated for 4 h at 37°C. The supernate was removed and the dark blue formazan complex was solubilized by adding 100 ul of 0.02 N HCl in 75% isopropanol to all wells. Absorbance at 490 nm was immediately determined using a scanning multiwell plate reader.

EXAMPLE X - Data

Table II below shows the effects of several second and third generation bradykinin antagonists on the cell growth of the SCLC cell line SHP-77 in an MTT assay. The second generation dimer CP-0127 and the second generation monomer B116 (HOE 140) had little effect on SCLC growth in concentration up to 40 μM. Likewise, a newly synthesized monomer termed B203 had little effect on SCLC growth in concentration up to 40 μM. In contrast, the homodimer of B203, termed B204, linked with a suberimido group through the $DArg^0$ amino acids produced 100% growth inhibition at 40 μM. Several other third generation dimers were studied (e.g., B168, B199, B201 and corresponding monomers, e.g., B202) and found to be highly effective in inhibiting the growth of SCLC cell lines. The most potent of these compounds (B199, B201) had IC_{50} concentrations ≤ 100 nM. (See Figures 1 and 2). The cytotoxicity was specific as these compounds produced no effect on the growth of fibroblasts and breast cancer cell lines at concentrations up to 10 μM. (Data not shown).

To understand the mechanism of the growth inhibition of these compounds, the effects on BK and cholecystokinin (CCK) induced signal transduction, SCLC growth and plasma stability were compared. The table below shows that the potency of the second (B116, CP-0127) and third generation (B194-B201) antagonists were similar for inhibition of BK induced calcium signaling (0.01 to 0.15 μM) and all nine tested did not inhibit signaling induced by CCK or gastrin. Many of the third generation compounds (B197-B204) were stable in plasma (>24 h), as was the second generation B116. Thus, plasma stability could not account for the increased growth inhibitory effects of many of the third generation dimers which had IC_{50}'s of about 100 nM for SCLC growth in MTT assays.

TABLE II

Effect of Bradykinin Antagonists on Calcium Flux in Response to BK and CCK, on Growth of SCLC Cell Lines in MTT Assay and on Stability in Plasma

Compound	IC_{50} (μM) Ca^{2+} (BK)	IC_{50} (μM) MTT	Plasma $t_{1/2}$ (hr)	% Inhibition CCK Ca^{2+}
B116	0.01	>40	>24	0
CP-0127	0.5	>40	<1	0
B168	.04	15	NT	NT
B194 9830HPLC 9452DMS9452	0.2	0.1	1	0
B195 9832HPLC 9452DSS9452	0.09	0.15	6	NT
B196 9834HPLC 9722BMH9722	0.07	0.4	1	8
B197 9836HPLC 9754BMH9754	0.05	0.2	>24	2
B198 9860HPLC3	0.15	0.10	>24	0
B199 9860HPLC4	0.08	0.08	>24	0
B200 9870HPLC1	0.02	1.0	>24	0
B201 9870HPLC2	0.02	0.15	>24	0
B202 9792(47-57)	0.1	>10	NT	NT
B203 9872HPLC2	0.03	>40	NT	NT
B204 9872HPLC3	0.008	35	NT	NT

NT = not tested

Without being bound by any particular theory regarding mechanism of action, these data indicate that these dimerized BK antagonists inhibit growth by a mechanism other than interruption of the calcium response such as induction of

apoptosis. Therefore, various SCLC cell lines were incubated with inhibitory concentrations of the dimerized BK antagonists and examined for their apoptotic effects. Dimeric compounds such as B197 induced an apoptotic response in SCLC cell lines whereas monomeric compounds such as HOE 140 (B116) produced no growth inhibition or apoptotic response. It has not yet been determined the mechanism of the apoptotic response (data not shown). However, it is suspected that the antagonists are binding to a non-peptide binding site on the peptide receptor and produce discordant signaling with activation of the matk/erk-kinase (MEKK) pathway.

All cited patents, patent documents and publications are incorporated by reference herein as though fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CORTECH, INC.

(ii) TITLE OF INVENTION: CYTOLYTIC BRADYKININ ANTAGONISTS

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) NAME: Schwegman, Lundberg,Woessner & Kluth
(B) STREET: 3500 IDS Center
(C) CITY: Minneapolis
(D) STATE: Minnesota
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 55402
(G) TELEPHONE: 612-339-0331
(H) TELEFAX: 612-339-3061

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: UNKNOWN
(B) FILING DATE: 03 SEPTEMBER 1996
(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: John E. Burke
(B) REGISTRATION NUMBER: 35,836
(C) REFERENCE/DOCKET NUMBER: 392.003WO1

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Arg Pro Pro Gly Phe Ser Pro Phe Arg
1               5
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Xaa Gly Lys Arg Pro Pro Gly Phe Ser Pro Leu
1               5                   10
```

CLAIMS

We claim:

1. A bradykinin antagonist compound of the general formula:

$$BKA_1\text{-}X\text{-}BKA_2,$$

wherein BKA_1 and BKA_2 are independently selected from the following:

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (SEQ ID NO:1);
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
ε-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Gun-Gly-ε-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:2);
Dhq-DArg-Arg-Pro-Hyp-Gly-ε-Lys-Ser-DCpg-CPg-Arg;
Dhq-ε-Lys-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg-Arg;
DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg;
DArg-Cys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;
DArg-Lys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;
DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-Tic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;
Gun- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;
Gun- DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;
Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;
Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

Lys- Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic; and

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic; or

BKA_2 is absent; and

X is a linker group.

2. The bradykinin antagonist according to claim 1 wherein X is a bissuccinimidoalkyl, bissuccinimidoalkenyl, bissuccinimidoamino, aminocaproic acid-succinyl, suberyl, bis(imidyl)alkyl, bis(imidyl)alkenyl, epsilon succinimido N-caproyl, or methoxy-suberimido linker.

3. The bradykinin antagonist according to claim 2 wherein X is BSH, BSD, SUIM or MOSI.

4. The bradykinin antagonist according to claim 2 wherein X comprises an alkyl group of 12 to 18 carbons.

5. The bradykinin antagonist according to claim 1 wherein BKA_1 and BKA_2 are N-terminally linked.

6. The bradykinin antagonist according to claim 1 wherein serine is replaced with cysteine or lysine and internally linked therethrough.

7. The bradykinin antagonist according to claim 1 selected from:

DArg-Arg-Pro-Hyp-Gly-Phe-Cys-DPhe-Phe-Arg-COOH

|

BSH

|

DArg-Arg-Pro-Hyp-Gly-Phe-Cys-DPhe-Phe-Arg-COOH;

DArg-Arg-Pro-Hyp-Gly-Igl-Cys-DIgl-Oic-Arg.TFA

|

DArg-Arg-Pro-Hyp-Gly-Igl-Cys-DIgl-Oic-Arg.TFA;

Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA
|
BSH
|
Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA;

Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.TFA
|
SUB
|
Gun-Gly-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu-desArg-COOH;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.TFA
|
SUIM
|
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.TFA;

Suc-(Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)$_2$;

Suc-(Eac-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)$_2$;

Suc-(Eac-Eac-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)$_2$;

Suim-(DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg)$_2$;

Sub-(DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg)$_2$;

BSH-(S-Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg)$_2$;

Dhq-DArg-Arg-Pro-Hyp-Gly-Lys-Ser-DCpg-CPg-Arg

|

Suc

|

Dhq-DArg-Arg-Pro-Hyp-Gly-Lys-Ser-DCpg-CPg-Arg; and

Suim-(DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg)$_2$.

8. The bradykinin antagonist according to claim 1 wherein BKA_1 and BKA_2 are selected from

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg, and

Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.

9. The bradykinin antagonist according to claim 8 wherein BKA_1-X-BKA_2 is

Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg

|

SUB

|

Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg; or

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg

|

SUIM

|

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg.

10. The bradykinin antagonist according to claim 1 wherein BKA_2 is absent.

11. The bradykinin antagonist according to claim 10 selected from

MOSI-Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

MOSI-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg; or

MOSI-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.

12. A compound according to the following:

BKA-X-Y;

wherein BKA is a bradykinin antagonist peptide selected from

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg;

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (SEQ ID NO:1);

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

ε-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

Gun-Gly-ε-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:2);

Dhq-DArg-Arg-Pro-Hyp-Gly-ϵ-Lys-Ser-DCpg-CPg-Arg;

Dhq-ϵ-Lys-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg-Arg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg;

DArg-Cys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

DArg-Lys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;

Gun- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;

Gun- DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;

Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;

Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic; and

Lys- Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

X is a linker; and

Y is neurokinin receptor antagonist.

13. The compound according to claim 12 wherein Y is selected from

Asp-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$;

Cys-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$;

DArg-DArg-Lys-Pro-Lys-Asn-DPhe-Phe-DTrp-Leu- (Nle);

p-HOPA-DTrp-Phe-DTrp-Leu-NH_2;

p-HOPA-DTrp-Phe-DTrp-Leu-$\Psi(CH_2NH)$Leu-NH_2;

DMePhe-DTrp-Phe-DTrp-Leu-$\Psi(CH_2NH)$Leu-NH_2;

DMePhe-DTrp-Tyr-DTrp-Leu-$\Psi(CH_2NH)$Leu-NH_2;

DTyr(Et)-DTrp-Phe-DTrp-Leu-$\Psi(CH_2NH)$Leu-NH_2;

DTyr-DTrp-Phe-DTrp-Leu-$\Psi(CH_2NH)$Leu-NH_2;

DMePhe-DTrp-Phe-DTrp-Leu-MPA; and

DMePhe-DTrp-Phe-DTrp-Leu-Leu-NH_2.

14. The compound according to claim 12 wherein

BKA is

Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg; or

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg; and

Y is DMePhe-DTrp-Phe-DTrp-Leu-$\Psi(CH_2NH)$Leu-NH_2.

15. A therapeutic method of inhibiting lung cancer cell growth comprising administering to a host in need of such treatment a therapeutically effective amount of one or more of the following compounds:

BKA_1-X-BKA_2	I;
BKA-X-Y	II;
BKA-X	III;
Y_1-X-Y_2	IV; or
$(BKA)_n$-X	V;

wherein BKA_1, BKA_2 and BKA are the same or different bradykinin antagonists,

X is a linker,

Y_1, Y_2 and Y are the same or different neurokinin receptor antagonist, and

n is a whole number greater than 2.

16. The method according to claim 15 wherein BKA_1, BKA_2 and BKA are peptides.

17. The method according to claim 16 wherein BKA_1, BKA_2, and BKA are selected from

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (SEQ ID NO:1);
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
ε-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Gun-Gly-ε-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:2);
Dhq-DArg-Arg-Pro-Hyp-Gly-ε-Lys-Ser-DCpg-CPg-Arg;
Dhq-ε-Lys-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg-Arg;
DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg;
DArg-Cys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;
DArg-Lys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;
DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-Tic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Cpg;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;
Gun- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;
Gun- DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;
Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;
Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
Lys- Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
DArg-Arg-Pro-Hyp-Gly-Phe-Cys-DPhe-Leu-Arg; and
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.

18. The method according to claim 17 wherein BKA_1, BKA_2, and BKA are selected from

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
ε-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic; and
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.

19. The method according to claim 18 wherein BKA_1-X-BKA_2 is

Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg
|
SUB
|
Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg
|
SUIM

|

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

MOSI-(DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic)$_2$; or

MOSI-(Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic)$_2$.

20. The method according to claim 15 wherein BKA_1 and BKA_2 are N-terminally linked.

21. The method according to claim 17 wherein serine, if present, is replaced with cysteine or lysine and internally linked therethrough.

22. The method according to claim 15 wherein X is a bissuccinimidoalkyl; bissuccunimidoalkenyl, bissuccinimidoamino, aminocaproic acid-succinyl, . suberyl, bis(imidyl)alkyl, bis(imidyl)alkenyl, epsilon succinimido N-caproyl, or methoxy-suberimido linker.

23. The method according to claim 15 wherein BKA-X is

MOSI-Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;
MOSI-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg; or
MOSI-DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg.

24. The method according to claim 15 wherein the compound is of the formula II and

BKA is selected from

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Oic-Arg;
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (SEQ ID NO:1);
DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Nig-Arg;
DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;
Cys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

ε-Lys-DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic-Arg;

Gun-Gly-ε-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (SEQ ID NO:2);

Dhq-DArg-Arg-Pro-Hyp-Gly-ϵ-Lys-Ser-DCpg-CPg-Arg;

Dhq-ϵ-Lys-DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg-Arg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DCpg-CPg;

DArg-Cys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

DArg-Lys-Pro-Hyp-Gly-Cpg-Ser-DCpg-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Tic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Cpg-Ser-DTic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DTic-Cpg;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Leu;

Gun- DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;

Gun- DArg-Arg-Pro-Hyp-Gly-Thi-Ser-DIgl-Oic;

DArg-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;

Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DTic-Cpg;

Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic; and

Lys- Lys-Arg-Pro-Hyp-Gly-Igl-Ser-DIgl-Oic;

and Y is selected from

Asp-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$;

Cys-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$;

DArg-DArg-Lys-Pro-Lys-Asn-DPhe-Phe-DTrp-Leu- (Nle);

p-HOPA-DTrp-Phe-DTrp-Leu-NH_2;

p-HOPA-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DMePhe-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DMePhe-DTrp-Tyr-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DTyr(Et)-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DTyr-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DMePhe-DTrp-Phe-DTrp-Leu-MPA; and

DMePhe-DTrp-Phe-DTrp-Leu-Leu-NH_2.

25. The method according to claim 15 wherein the compound is of the formula IV and Y_1 and Y_2 are selected from

Asp-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$;

Cys-Tyr-DTrp-Val-DTrp-DTrp-Arg-$CONH_2$;

DArg-DArg-Lys-Pro-Lys-Asn-DPhe-Phe-DTrp-Leu- (Nle);

p-HOPA-DTrp-Phe-DTrp-Leu-NH_2;

p-HOPA-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DMePhe-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DMePhe-DTrp-Tyr-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DTyr(Et)-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DTyr-DTrp-Phe-DTrp-Leu-Ψ(CH_2NH)Leu-NH_2;

DMePhe-DTrp-Phe-DTrp-Leu-MPA; and

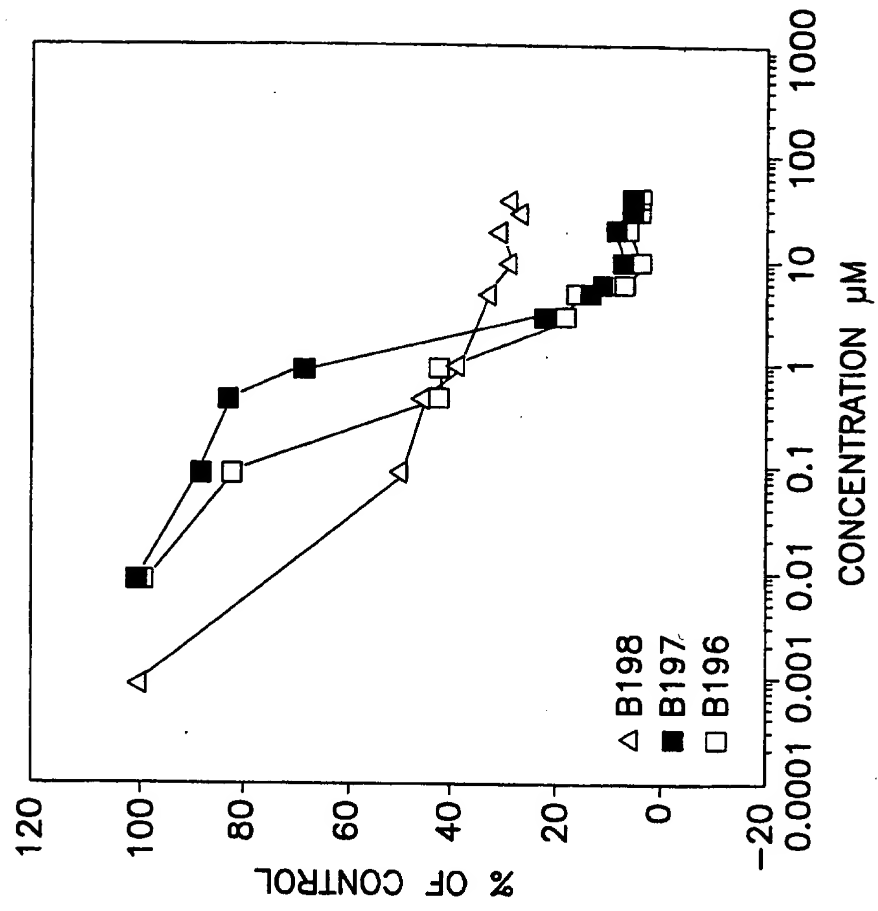
DMePhe-DTrp-Phe-DTrp-Leu-Leu-NH_2.

26. The method according to claim 15 wherein the compound is of the formula V and n is 3.

27. The method according to claim 15 wherein the cancer cells are small cell lung carcinoma.

28. The method according to claim 15 wherein the bradykinin antagonist is administered via intratracheal instillation.

29. The method according to claim 15 wherein the bradykinin antagonist is administered via aerosol inhalation.



% OF CONTROL
120
100
80
60
40
20
0
−20
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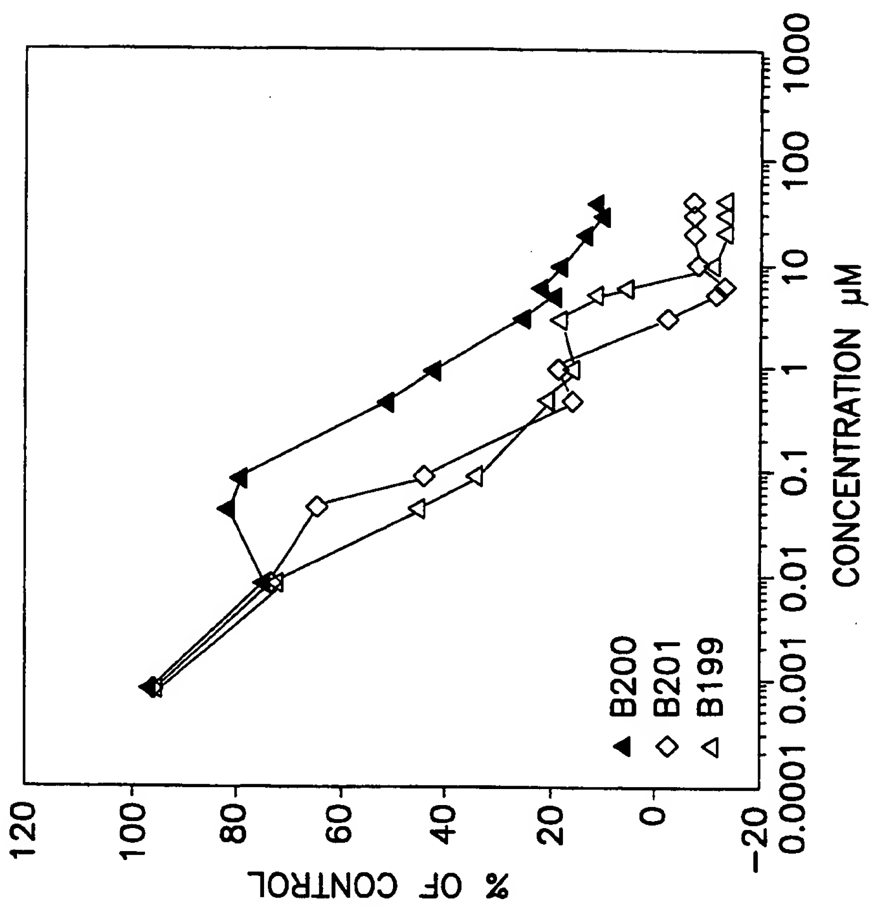
0.001
0.01
0.1
1
10
100
1000
CONCENTRATION μM
B198
B197
B196

FIG. 1


% OF CONTROL
CONCENTRATION µM
B200
B201
B199
120
100
80
60
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20
0
−20
0.0001
0.001
0.01
0.1
1
10
100
1000

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/14113

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K7/18 C07K19/00 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 17201 (CORTECH INC) 15 October 1992 see claims 1-28; tables F,K,N,R ---	1-10,12, 13
X	H.FRITZ: "Proceedings Int.Conf. "Kinin 81 Munich", 1981" XP002018556 J.M.Stewart e.a.: Bradykinin chemistry: agonists and antagonists" see page 585 - page 589 ---	1,5-7,10

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
14 November 1996	22. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Groenendijk, M

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GROWTH FACTORS, vol. 5, 1991, pages 159-170, XP002018550 S.L.GAWLAK E.A.: "Homodimeric forms of bombesin act as potent antagonists of bombesin on Swiss 3T3 cells." The whole document; see especially page 166, col.2 to page 167, col.1; fig.8; page 168, col. 2	15,27
P,X	PROC.87TH ANNUAL MEETING AM.ASS.CANCER RES. APRIL, 1996, vol. 37, March 1996, page 417 XP002018551 Abstr. Nos. 2844 and 2845	1-29
P,X	IMMUNOPHARMACOLOGY, vol. 33, no. 1-3, 1996, pages 178-182, XP002018552 L GERA E.A: "A new class of bradykinin antagonist dimers" see the whole document	1-29
P,X	IMMUNOPHARMACOLOGY, vol. 33, no. 1-3, 1996, pages 201-204, XP002018553 D.CHAN E.A.: "Novel bradykinin antagonist dimers for the treatment of human lung cancers" see the whole document	1-29
A	CANCER RESEARCH (SUPPL.), vol. 52, 1 May 1992, MD US, pages 2737s-2742s, XP002018554 T.SETHI E.A.: "Growth of SCLC's: Stimulation by multiple neuropeptides and inhibition by broad spectrum antagonists in vitro and in vivo" see the whole document	1-29
A	CANCER RESEARCH, vol. 54, 1994, MD US, pages 3602-3610, XP002018555 P.A.BUNN E.A.: "Effects of neuropeptide analogues on calcium flux and proliferation in lung cancer cell lines" cited in the application see the whole document	1-29

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/14113

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9217201	15-10-92	AU-B- 660683	06-07-95
		AU-A- 1875192	02-11-92
		CA-A- 2106677	02-10-92
		CZ-A- 9302036	13-07-94
		EP-A- 0586613	16-03-94
		HU-A- 65328	02-05-94
		JP-T- 6508116	14-09-94
		SK-A- 106193	11-05-94
		US-A- 5416191	16-05-95

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 96/14113

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 15-29
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 15-29 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.